Study On Infectious Bovine Rhinotracheitis Virus Infection In Calves

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ABESTRACT

This study was applied to investigate the effect of infectious bovine rhinotracheitis virus on the young calves. Four hundred twenty two Holstein calves aged from one to 18 months were examined during the period from May 2009 to March 2010. The clinical examination revealed that 109 (25.8%) animals. These calves were suffered from respiratory signs. Calves were found in different farms in Zagazig and 10th of Ramadan - Sharkia Gevernorate. IBR (BHV-1) virus was isolated on the MDBK cell line and identified with virus neutralization test from 19 (17.4%) out of 109 collected nasal swabs. Antibodies against IBR were detected in 53 (48.6%) out of 109 collected serum samples and tested by ELISA test.

INTRODUCTION

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) is a significant disease among domestic and wild bovine animals *(1,2)*. Clinical signs of the infection include symptoms of inflammatory processes on both respiratory and genital organs, and abortion. Young calves can develop a systemic disease affecting visceral organs *(3)*. The disease is caused by the bovine herpesvirus 1 (BHV-1), in the subfamily Alphaherpesvirinae of the *Herpesviridae* family.

Only a single serotype of BHV-1 is recognized, but subtypes of BHV-1 have been described on the basis of restriction enzyme cleavage patterns of viral DNA. These types are referred to as BHV-1.1 (respiratory subtype), BHV-1.2 (respiratory and genital subtype) *(4)*. BHV-1 is able to establish a latent infection in the trigeminal or sacral ganglia *(5)*. Animals with a latent BHV-1 infection may serve as a source of infection for susceptible animals if and when the virus is reactivated *(6)*. The infection is transmitted mainly through respiratory, ocular or genital secretions in a direct contact between animals.

However, the infection can also spread through fresh or frozen semen from infected bulls *(7)*. The disease is notifiable in many, but not all countries. Common control measures against IBR/IPV include screening, surveillance, precautions at borders and a modified stamping out policy. Many countries allow and carry out vaccinations, while vaccination is prohibited in countries that have eradicated the disease *(2)*.

In Egypt, the papers who deals with IBR virus infection in calves still few and not dealing completely with this problem. In spite of some few paper which had been published *(8-11)*.

The aim of this study is directed toward how the infection with IBR virus affecting economically on the growth and production of young growing cattle which well be used in the beef and dairy production through the following steps:

1- Trails for isolation and identification of IBR virus in the collected nasal swabs from the affected animals.

2- Detection of the antibodies against IBR virus in the collected serum samples which taken from the affected animals.

MATERIAL AND METHODS

1- Material

a- Animals

Four hundreds and twenty two (422) Holstein calves are examined in the present study. The examination revealed that 109 animals were found with respiratory signs. These calves were found in different farms in Zagazig and 10th of Ramadan - Sharkia Gevernorate. The affected animals are tested

from May 2009 to March 2010 in which the samples were collected.

b- Samples

1- Nasal swabs

One hundred and nine (109) nasal swabs were collected from the affected cattle in the early phase of the infection. These cattle still have serous nasal discharge. Sterile swab was inserted in nasal opening as far as possible to avoid the contamination with mucous discharge. Each swab was immersed in sterilized tube containing phosphate buffer saline (pH 7.2) and kept in laboratory at -70 °C until used . The collected swabs were used for virological investigations.

2- Serum samples

One hundred and nine (109) blood samples were collected from jugular vein using a sterile needle for each animal in a sterile labeled venoject tube. The collected samples were taken from the affected animals 2 weeks after taking the nasal swabs. All tubes were transported as early as possible on an ice packed thermos to the laboratory where they were centrifuged at 2000 r.p.m for 10 minutes to obtain clear serum samples. The sera were separated in a sterile capped vials and inactivated at 56°C for 30 minutes to remove non specific inhibitors and preserved at -70°C tell used.

b- Phosphate buffer saline

This solution was prepared *(12)* and autoclaved at 15 lb pressure for 15 minutes and used in preparation of collected nasal swab samples.

c- Cell culture

A continuous cell line of Madian Darby Bovine kidney (MDBK) cells were Supplied by Rinderpest like Disease Department, Veterinary Serum and Vaccine Research Institute, Abassia – Cairo. The cells were grown and propagated using modified Egle's minimum essential medium (EMEM). The MDBK cells were used for virus isolation and identification.

d- Reference IBR virus and antiserum

They were kindly supplied from the Rinderpest like Diseases Department, Veterinary Serum and Vaccine Research Institute – Abbasia, Cairo – and was used in VNT and ELISA tests.

e- ELISA kits

It was supplied from IDEXX Laboratories Inc. One IDEXX Drive, Westbrook, Maine 04092, USA..

3- Methods

a- Detection of IBR virus in tissue culture cell lines

The virus was isolated according to the previously described protocol *(13)*. Isolation attempts were done using 109 samples in trail to isolate IBR virus on MDBK cells. MDBK cells were distributed in plastic 96 wells tissue culture plate for 70% confluence, the growth media was discarded and 50 µl from each sample was inoculated into triplicate wells. For each plate, cell control and virus control were included. The plates were incubated for one hour for adsorption, the virus inoculum and the plates was washed using 200µl of EMEM added to each well, then incubated at 37°C for 3-5 days with daily examination for recording the development of cytopathic effect. The virus was harvested and used for subsequent passages. After the 3rd passage, the cells were harvested and kept at – 70 °C for virus identification.

b- Identification of the isolated virus

The identification was done by using Virus neutralization test *(14)*.

c- Detection of antibodies against IBR virus

This was done using ELISA test in the collected serum samples from the affected calves according to the method described by IDEXX Laboratories Inc. One IDEXX Drive, Westbrook, Maine 04092, USA..

RESULTS

Clinical examination of 422 calves in different farms in Zagazig and 10th of Ramadan, Sharkia Governorate revealed that 109 (25.8%) (Table 1) calves were suffered from coughing, sneezing, presence of bilateral nasal discharge in the form of serous, mucoid and sometimes mucopurulent in nature. Body temperatures ranged from 39-40.5°C (Fig.1).

Trails for virus isolation on MDBK tissue culture cell lines, and with daily observation of the injected tissue culture for 3-5 days. The results revealed that 19 (17.4%) out of 109 swab samples giving positive results. The positive samples were showed characteristic cytopathoic effect (CPE) appeared as a characteristic grape like clusters which are rounded and aggregated together in a separate manner. The CPE appearded after 3-5 days post inoculation in the first passage and gradually increased till 70-80% of the sheet was completely detached in some samples (Table 2 & Fig. 2). Identification of the isolated virus by using virus neutralization test revealed that the isolated virus was infectious bovine rhinotracheitis virus (BHV-1).

Detection of antibodies against infectious bovine rhinotracheitis (IBR) virus in the collected serum samples by using ELISA test revealed that 53 (48.6%) out of 109 serum samples are giving positive results (Table 3).

Table 1. Results of the clinical examination.

Age (month)	No. of examined animals	Clinically affected animals	%
1 – 6	117	42	35.9
7 – 12	135	38	28.1
13 – 18	170	29	17.1
Total	422	109	25.8

Table 2. Results of virus isolation in tissue culture.

Age (month)	Collected nasal swabs	Positive swabs	%
1 – 6	42	10	23.8
7 – 12	38	6	15.8
13 – 18	29	3	10.3
Total	109	19	17.4

Table 3. Detection of antibodies against IBR virus using ELISA test.

Age (month)	Collected serum samples	Positive samples	%
1 – 6	42	23	54.8
7 – 12	38	19	50
13 – 18	29	11	37.9
Total	109	53	48.6

Fig. 1.Animal clinically affected with IBR showed lacrimation and bilateral nasal discharge.

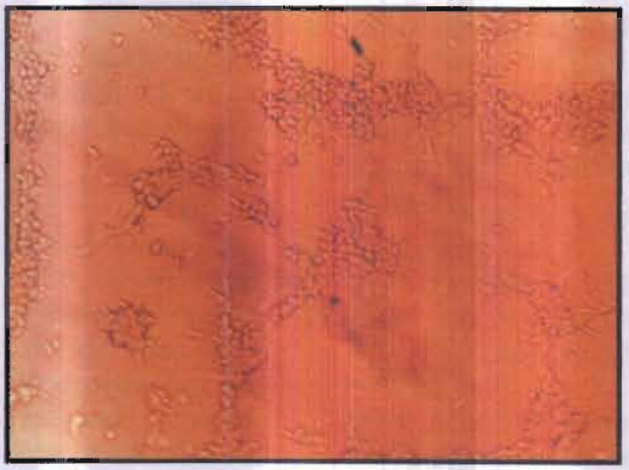

Fig. 2. MDBK cell lines infected with IBR virus (grapes like appearance).

DISCUSSION

Infectious bovine rhinotracheitis (IBR) virus is a member of the *Alphaherpesvirinae* subfamily. It is responsible for a variety of clinical syndromes in cattle, including respiratory disorders, conjunctivitis, genital infections, encephalitis, abortions, and a multisystemic fatal disease in neonates. Acute infection of the respiratory tract by IBR can induce immunosuppression, which predisposes cattle to secondary bacterial colonization, severe pneumonia, and even death ***(15)***. The main sources of infection are the nasal exudates and the respiratory droplets, genital secretions, semen, fetal fluids and tissues ***(16)***.

In the present study, clinical examination of 422 Holstein calves revealed that 109 (25.8%) calves were suffered from respiratory signs in the form of coughing, sneezing, presence of bilateral nasal discharge in the form of serous, mucoid and sometimes mucopurulent in nature. Body temperatures were ranged from 39-40.5°C.

The clinical signs recorded in this study were nearly similar to several previous studies ***(10, 17 ,18)*** who reported that the respiratory form of IBR manifested by fever, reduced appetite, rapid respiration dyspnea, nasal discharge and dilated nostrils.

Trails for virus isolation on MDBK cell line from the collected nasal swabs revealed that 19 (17.4%) swabs out of 109 collected swabs showed positive results (Table 2) The positive samples showed characteristic cytopathoic effect (CPE) appeared as characteristic grape like clusters which are rounded and aggregated together in a separate manner. The CPE occurred after 3-5 days post inoculation and gradually increased till 70-80% of the sheet was completely detached in some samples.

Identification of the isolated virus was carried out using virus neutralization test (VNT) against reference hyperimmune serum of IBR virus.

IBR was isolated in MDBK cell lines from outbreak of conjunctivitis in a cattle herd of 581 cows and calves ***(11)*** it has been isolated from two nasal swabs collected from calves with upper respiratory tract syndrome ***(19)***.

On the other hand, failure of isolation of IBR virus (BHV-1) was recorded ***(20)*** were IBR virus failed to be isolated on MDBK cell lines from nasal swabs collected from affected calves

Detection of antibodies against IBR virus (BHV-1) in the collected serum samples from affected calves two weeks after collection nasal swabs using of ELISA test revealed that 53 (48.6%) out of 109 serum samples are giving positive results (Table 3).

Antibodies against IBR virus was detected in 17 (48.6%) out of 35 serum samples collected from affected calves and tested by ELISA test ***(21)***, and it has been reported positive antibodies against IBR virus in 48 (54.5%) out of 88 serum samples collected from affected calves and tested by ELISA test ***(22)***.

On the other hand, higher IBR (BHV-1) antibodies titers results were recorded in previous studies ***(23, 24, 25)*** which detected antibodies against IBR virus (97.7%, 89% and 78% respectively) in the collected serum samples from affected calves with upper respiratory tract affection and test by ELISA test.

Lower results were recorded which detected antibodies against IBR virus (13.78% and 8% respectively) in the collected serum samples from affected calves with respiratory signs ***(20,26)***.

CONCLUSION

From our study, IBR represent a problem in the cattle population because it affect on the growth and production of the affected animals as well as it playing an important role in the secondary bacterial invasion by *Pasteurella Spp.* So, vaccinal programs against IBR should be applied to contrl the incidence of infection.

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الملخص العربي

دراسات عن فيروس التهاب الأنف و القصبة الهوائية المعدي في العجول البقرية

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أجريت هذه الدراسة خلال الفترة من مايو ٢٠٠٩ إلى مارس ٢٠١٠ لبيان تأثير مرض التهاب الأنف و القصبة الهوائية المعدي للأبقار على العجول الصغيرة. تم الفحص الإكلينيكي لعدد ٤٢٢ من عجول الهالوشتين البقرية (في عدة مزارع بمنطقتي الزقازيق و العاشر من رمضان بمحافظة الشرقية) تراوحت أعمارهم من شهر واحد إلى ١٨ شهر. أسفرت الفحوصات عن وجود عدد ١٠٩ (٢٥,٨%) عجل بقري عليهم الأعراض التنفسية تم تجميع عدد ١٠٩ عينات مسحات أنفية و ١٠٩ عينة من مصل الدم من هذه الحيوانات. و قد أسفرت محاولات عزل الفيروس عن وجود ١٩ (١٧,٤%) من المسحات الأنفية موجبة تم التأكد منها بإستخدام اختبار التعادل الفيروسي لفيروس مرض التهاب الأنف و القصبة الهوائية المعدي للأبقار, كما تم الكشف عن وجود أجسام مناعية في ٥٣ (٤٨,٦%) من عينات أمصال الدم باستخدام إختبار الإليزا.